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- Duchenne muscular dystrophy (DMD) is a neuromuscular disorder characterized by progressive muscle weakness resulting from truncated, non-functional dystrophin protein^{1,2}
- BMN 351 (nivudirsen) is an exon 51-skipping antisense oligonucleotide (ASO) designed to enable synthesis of functional, near full-length dystrophin protein^{3,4}
- Study 351-201 (EUCT#2023-506737-30-00; NCT06280209) is an ongoing, open-label, phase 1/2 clinical trial of nivudirsen in participants with DMD amenable to exon 51 skipping
- Measurement of ASO drug concentration and localization in muscle tissue provides pharmacokinetic (PK) data
- Exon skipping, near full-length dystrophin protein, and gene expression biomarkers are pharmacodynamic (PD) measures of nivudirsen activity from muscle biopsies
- Systemic muscle injury biomarkers are global PD readouts from longitudinal serum and urine samples

- The objective of this poster is to describe a multifaceted approach to understand drug exposure, mechanism of action, and impact on muscle gene expression and systemic biomarkers of muscle injury resulting from nivudirsén treatment
- Results from clinical study 351-201 are presented in additional posters by BioMarin Pharmaceutical Inc. (see QR codes)

- Muscle biopsy samples were collected from the biceps of participants in the 351-201 trial at baseline and after 13 or 25 weekly nivudirsén administrations
- The concentration of nivudirsén was measured in muscle biopsy tissue using a qualified quantitative hybridization ELISA (**Figure 1**)

Anti-DIG-HRP

Nivudirsen TEG — **G G U A A G U U C U G U C C A A G C**

Biotin — **C C A T T C A A G** — **A C A G G T T C G** — **DIG**

Biotin-capture oligo **DIG-detection oligo**

DIG, dioxynovinin; HRP, horseradish peroxidase; oligo, oligonucleotide; TEG, triethylene glycol

- The distribution of nivudirsin in muscle was characterized by exploratory miRNAScope fluorescence in situ hybridization (FISH)
- *DMD* mRNA exon 51 skipping was measured in muscle using a characterized droplet digital PCR (ddPCR) method (**Figure 2**)

- Near full-length dystrophin protein was measured in muscle by quantitative immunoaffinity liquid chromatography-tandem mass spectrometry (LC-MS; **Figure 3**)

Diagram illustrating the structure of the human IL-13 protein, showing the N-terminal domain, Rod-shaped domain, Cysteine-rich domain, and C-terminal domain. The protein is composed of 79 amino acids. Key regions include the ABD1 domain (N-terminal), the LLQ peptide for quantification (residues 48-50), the H1, H2, H3, and H4 domains, the CR domain (Cysteine-rich), and the ZF domain (C-terminal). The diagram also indicates the capture antibody binding site (residues 60-70) and the patient deletion site (residues 48-50).

- Muscle RNA sequencing data were generated from wild-type or hMDMDel52/*mdx* mice treated for 13 weeks with vehicle or nivodirsen (18 mg/kg administered weekly)³
 - RNA sequencing was analyzed using a custom Snakemake pipeline; alignment and gene-level quantification were determined using STAR. Differential expression was assessed with DESeq2, and principal components analyses and gene set variation analyses were performed in R using base stats and the Gene Set Variation Analysis (GSVA) packages
- Myoblast- and myofiber-expressed genes were measured by quantitative ddPCR and used to calculate a myoblast-to-myofiber gene expression ratio
- Muscle function in mice was measured by MotoRater kinematic gait analysis
- Lactate dehydrogenase (LDH) and creatine kinase (CK) were measured in serum using validated enzymatic colorimetry assays
- Titin was measured in urine by ELISA and normalized to osmolality

Nivirdisren dose (once weekly)	Nivirdisren skeletal muscle concentration (nmol/g)	n
6 mg/kg	4.39	4
9 mg/kg	6.53	6

Data are reported as the mean $\pm$ standard error of the mean for week 25 biopsies to facilitate direct comparison of the nivudirsene concentration (cohort 1A 6-mg/kg biopsies collected at week 13 were not included in the analysis).

Nivirdirsin (red), WGA (green), DAPI (blue)

Nivirdirsin (red), DAPI (blue)

Nivirdirsin in extracellular space

Nivirdirsin in nuclei

Nivirdirsin in myofibers

Scatter plot showing the relationship between Exon 51-skipped *DMD*/*GAPDH* mRNA (X-axis) and LC-MS dystrophin protein levels (Y-axis). The Y-axis is labeled 'LC-MS dystrophin (pM)/total protein (µg/mL)' and ranges from 0.00 to 0.10. The X-axis is labeled 'Exon 51-skipped *DMD*/*GAPDH* mRNA' and ranges from 0.00 to 0.06. Data points are categorized by 'Baseline' (open circles) and 'Post-dose' (filled squares). The plot shows a positive correlation, with post-dose levels generally higher than baseline levels for the same mRNA levels.

DMD, dystrophin gene; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; LC-MS, immunoaffinity ultra-performance liquid chromatography coupled with tandem mass spectrometry.

351-201
clinical data



- Disease-associated gene expression in DMD mice involved muscle regeneration, extracellular matrix, and adaptive immune pathways (**Figure 7**)
- Muscle gene expression in DMD mice treated with nivudirsen for 13 weeks reverted to wild-type, suggesting broad impact on muscle biology

Heatmap showing scaled expression (color scale: -4 to 4) of 20 genes across three conditions: WT (vehicle-treated), hDMDdel52/mdx (vehicle-treated), and hDMDdel52/mdx (nivudirsen-treated). The genes are grouped into three categories: Muscle regeneration, ECM/fibrosis, and Adaptive immune.

Gene Category	Gene	WT (vehicle-treated)	hDMDdel52/mdx (vehicle-treated)	hDMDdel52/mdx (nivudirsen-treated)
Muscle regeneration	Quiescence	Low	Low	Low
	Early activation	Low	High	Low
	Differentiation	Low	High	Low
	Fusion	Low	High	Low
	Immature fibers	Low	High	Low
	Mature myofiber	Low	High	Low
	Functional coupling	Low	Low	Low
	Structural/metabolic	Low	Low	Low
ECM/fibrosis	Regulator	Low	High	Low
	Collagen synthesis	Low	High	Low
	Collagen maturation	Low	High	Low
	ECM deposition	Low	High	Low
	ECM degradation	Low	High	Low
	Structure (BM)	Low	High	Low
Adaptive immune	Monocyte recruitment	Low	High	Low
	Inflammation	Low	High	Low
	Macrophage	Low	High	Low
	Damage sensing	Low	High	Low
	Fibrosis driver	Low	High	Low
	T cells	Low	High	Low

Heatmaps in Figure 7 were generated using variance-stabilized expression values from an unsupervised DESeq2 model. DMD, Duchenne muscular dystrophy; ECM, extracellular matrix; WT, wild-type.

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- Scatter plot showing Myoblast/myofiber expression ratio $\log_{10}$ scale (Y-axis) versus Gait score (function) (X-axis). The Y-axis is logarithmic, ranging from 0.1 to 100. The X-axis ranges from -4 to 12. Data points are categorized by color and shape: grey circles for WT (vehicle-treated), red circles for hDMDdel52/mdx (vehicle-treated), and blue circles for hDMDdel52/mdx (nivudirsen-treated).
- | Gait score (function) | Myoblast/myofiber expression ratio $\log_{10}$ scale | Group |
|-----------------------|--|------------------------------------|
| -3.5 | 0.5 | WT (vehicle-treated) |
| -2.5 | 1.0 | WT (vehicle-treated) |
| -1.5 | 0.25 | WT (vehicle-treated) |
| 0.5 | 1.2 | WT (vehicle-treated) |
| 0.5 | 2.5 | WT (vehicle-treated) |
| 1.5 | 0.9 | WT (vehicle-treated) |
| 1.5 | 2.5 | hDMDdel52/mdx (nivudirsen-treated) |
| 2.5 | 2.0 | hDMDdel52/mdx (nivudirsen-treated) |
| 3.5 | 2.2 | hDMDdel52/mdx (nivudirsen-treated) |
| 3.5 | 12 | hDMDdel52/mdx (vehicle-treated) |
| 3.8 | 60 | hDMDdel52/mdx (vehicle-treated) |
| 4.0 | 12 | hDMDdel52/mdx (vehicle-treated) |
| 4.2 | 10 | hDMDdel52/mdx (vehicle-treated) |
| 10.5 | 50 | hDMDdel52/mdx (vehicle-treated) |

Gene expression ratio is calculated from 3 genes each expressed primarily in myoblasts or myofibers, relative to WT expression levels. Gait score was determined using fine motor kinematic testing; lower gait score indicates higher function.³
DMD, Duchenne muscular dystrophy; WT, wild-type.

- Serum LDH, serum CK, and urinary titin are elevated in untreated individuals with DMD compared with the expected range in a healthy pediatric population, reflecting muscle damage (**Figure 9**)

A) Serum lactate dehydrogenase (U/L) for 351-201 baseline. The y-axis ranges from 0 to 1800 U/L. Individual data points are shown as orange dots. Horizontal dashed lines indicate reference values at approximately 150, 350, and 370 U/L.

B) Serum creatine kinase (U/L) for 351-201 baseline. The y-axis ranges from 0 to 40,000 U/L. Individual data points are shown as orange dots. Horizontal dashed lines indicate reference values at approximately 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900, 3000, 3100, 3200, 3300, 3400, 3500, 3600, 3700, 3800, 3900, 4000, 4100, 4200, 4300, 4400, 4500, 4600, 4700, 4800, 4900, 5000, 5100, 5200, 5300, 5400, 5500, 5600, 5700, 5800, 5900, 6000, 6100, 6200, 6300, 6400, 6500, 6600, 6700, 6800, 6900, 7000, 7100, 7200, 7300, 7400, 7500, 7600, 7700, 7800, 7900, 8000, 8100, 8200, 8300, 8400, 8500, 8600, 8700, 8800, 8900, 9000, 9100, 9200, 9300, 9400, 9500, 9600, 9700, 9800, 9900, 10000, 10100, 10200, 10300, 10400, 10500, 10600, 10700, 10800, 10900, 11000, 11100, 11200, 11300, 11400, 11500, 11600, 11700, 11800, 11900, 12000, 12100, 12200, 12300, 12400, 12500, 12600, 12700, 12800, 12900, 13000, 13100, 13200, 13300, 13400, 13500, 13600, 13700, 13800, 13900, 14000, 14100, 14200, 14300, 14400, 14500, 14600, 14700, 14800, 14900, 15000, 15100, 15200, 15300, 15400, 15500, 15600, 15700, 15800, 15900, 16000, 16100, 16200, 16300, 16400, 16500, 16600, 16700, 16800, 16900, 17000, 17100, 17200, 17300, 17400, 17500, 17600, 17700, 17800, 17900, 18000, 18100, 18200, 18300, 18400, 18500, 18600, 18700, 18800, 18900, 19000, 19100, 19200, 19300, 19400, 19500, 19600, 19700, 19800, 19900, 20000, 20100, 20200, 20300, 20400, 20500, 20600, 20700, 20800, 20900, 21000, 21100, 21200, 21300, 21400, 21500, 21600, 21700, 21800, 21900, 22000, 22100, 22200, 22300, 22400, 22500, 22600, 22700, 22800, 22900, 23000, 23100, 23200, 23300, 23400, 23500, 23600, 23700, 23800, 23900, 24000, 24100, 24200, 24300, 24400, 24500, 24600, 24700, 24800, 24900, 25000, 25100, 25200, 25300, 25400, 25500, 25600, 25700, 25800, 25900, 26000, 26100, 26200, 26300, 26400, 26500, 26600, 26700, 26800, 26900, 27000, 27100, 27200, 27300, 27400, 27500, 27600, 27700, 27800, 27900, 28000, 28100, 28200, 28300, 28400, 28500, 28600, 28700, 28800, 28900, 29000, 29100, 29200, 29300, 29400, 29500, 29600, 29700, 29800, 29900, 30000, 30100, 30200, 30300, 30400, 30500, 30600, 30700, 30800, 30900, 31000, 31100, 31200, 31300, 31400, 31500, 31600, 31700, 31800, 31900, 32000, 32100, 32200, 32300, 32400, 32500, 32600, 32700, 32800, 32900, 33000, 33100, 33200, 33300, 33400, 33500, 33600, 33700, 33800, 33900, 34000, 34100, 34200, 34300, 34400, 34500, 34600, 34700, 34800, 34900, 35000, 35100, 35200, 35300, 35400, 35500, 35600, 35700, 35800, 35900, 36000, 36100, 36200, 36300, 36400, 36500, 36600, 36700, 36800, 36900, 37000, 37100, 37200, 37300, 37400, 37500, 37600, 37700, 37800, 37900, 38000, 38100, 38200, 38300, 38400, 38500, 38600, 38700, 38800, 38900, 39000, 39100, 39200, 39300, 39400, 39500, 39600, 39700, 39800, 39900, 40000, 40100, 40200, 40300, 40400, 40500, 40600, 40700, 40800, 40900, 41000, 41100, 41200, 41300, 41400, 41500, 41600, 41700, 41800, 41900, 42000, 42100, 42200, 42300, 42400, 42500, 42600, 42700, 42800, 42900, 43000, 43100, 43200, 43300, 43400, 43500, 43600, 43700, 43800, 43900, 44000, 44100, 44200, 44300, 44400, 44500, 44600, 44700, 44800, 44900, 45000, 45100, 45200, 45300, 45400, 45500, 45600, 45700, 45800, 45900, 46000, 46100, 46200, 46300, 46400, 46500, 46600, 46700, 46800, 46900, 47000, 47100, 47200, 47300, 47400, 47500, 47600, 47700, 47800, 47900, 48000, 48100, 48200, 48300, 48400, 48500, 48600, 48700, 48800, 48900, 49000, 49100, 49200, 49300, 49400, 49500, 49600, 49700, 49800, 49900, 50000, 50100, 50200, 50300, 50400, 50500, 50600, 50700, 50800, 50900, 51000, 51100, 51200, 51300, 51400, 51500, 51600, 51700, 51800, 51900, 52000, 52100, 52200, 52300, 52400, 52500, 52600, 52700, 52800, 52900, 53000, 53100, 53200, 53300, 53400, 53500, 53600, 53700, 53800, 53900, 54000, 54100, 54200, 54300, 54400, 54500, 54600, 54700, 54800, 54900, 55000, 55100, 55

Points represent baseline results, and dotted lines represent the range expected for a healthy pediatric population aged 4 to 9 years.
DMD, Duchenne muscular dystrophy

Biomarkers of muscle injury



- Together, the data from this suite of measures uniquely provide a deep and holistic understanding of the PK and PD of nivudirsén
- These assays have been leveraged in preclinical and clinical studies to understand the molecular and cellular changes resulting from nivudirsén treatment
- The preclinical and clinical data presented here, together with the subsequent ongoing phase 1/2 study, support the potential for benefit from nivudirsén treatment in individuals with DMD

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PK/PD of
nivudirsen



Biomarkers of muscle injury

351-201
clinical data

